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Short communication

Anaerobic degradation of dimethylsulfoniopropionate to 3-*S*-methylmercaptopropionate by a marine *Desulfobacterium* strain

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Abstract. Dimethylsulfoniopropionate, an osmolyte of marine algae, is thought to be the major precursor of dimethyl sulfide, which plays a dominant role in biogenic sulfur emission. The marine sulfate-reducing bacterium *Desulfobacterium* strain PM4 was found to degrade dimethylsulfoniopropionate to 3-*S*-methylmercaptopropionate. The oxidation of one of the methyl groups of dimethylsulfoniopropionate was coupled to the reduction of sulfate; this process is similar to the degradation of betaine to dimethylglycine which was described earlier for the same strain. *Desulfobacterium* PM4 is the first example of an anaerobic marine bacterium that is able to demethylate dimethylsulfoniopropionate.

Key words: Anaerobic degradation — Methylated sulfur compounds — Dimethylsulfoniopropionate — 3-*S*-Methylmercaptopropionate — Marine sulfate-reducing bacterium

Dimethylsulfoniopropionate (DMSP) is one of the major sulfonium compounds in the marine environment and is present in many algae, e.g. *Emiliania huxleyi*, and *Phaeocystis* sp. (Reed 1983; Keller 1989). DMSP has also been found in the cyanobacterium *Microcoleus chthonoplastes* (Visscher and van Gernerden 1991). In these organisms DMSP probably acts as a compatible solute for osmoregulation (Kirst 1989). On a global scale DMSP is thought to be the most important source of dimethyl sulfide (DMS), which plays a dominant role in marine biogenic sulfur emission. DMS contributes, through its atmospheric oxidation products sulfuric acid and methanesulfonic acid, to acid precipitation and cloud formation, and thus to climate regulation (Charlson et al. 1987).

In anoxic coastal sediments DMSP is either cleaved to DMS and acrylate or demethylated to 3-*S*-methyl-

mercaptopropionate (MMPA) and subsequently to 3-mercaptopropionate (Kiene and Taylor 1988a, b). Aerobically, DMSP may also undergo initial cleavage to DMS and acrylate. Certain aerobic bacteria were shown to degrade DMSP via alternative pathway(s) presumably with MMPA and 3-mercaptopropionate as intermediates (Taylor and Gilchrist 1991). The only anaerobic bacterium that was reported to metabolize DMSP is a *Clostridium* strain that was isolated from river mud; it cleaved DMSP to DMS and acrylate (Wagner and Stadtman 1962). Thus far, however, no anaerobic DMSP-metabolizing bacteria have been described from marine environments.

Here we report on the demethylation of DMSP by *Desulfobacterium* sp. strain PM4, which had originally been isolated from anoxic marine sediment with betaine as a substrate (Heijthuijsen and Hansen 1989b). Strain PM4 degrades betaine to *N,N*-dimethylglycine. Betaine, like DMSP, is an important osmoregulatory solute and structurally resembles DMSP. It was for this reason that we investigated whether strain PM4 is able to degrade DMSP.

Materials and methods

Organism and cultivation

Desulfobacterium sp. strain PM4 was cultivated in a mineral medium (pH 6.9) with yeast extract (0.02%) and Na₂SO₄ (20 mM) according to Heijthuijsen and Hansen (1989a). Incubations with DMSP were carried out in completely filled crimp seal bottles at 28 °C. The butyl rubber stoppers were pretreated by boiling in 0.1 M NaOH to remove contaminating sulfur compounds. DMSP was added from a filter-sterilized solution (in 1 M NaHCO₃) to the desired concentration (up to 20 mM).

Analytical procedures

Sulfide was measured colorimetrically (Trüper and Schlegel 1964). DMSP was determined as DMS, after treatment with 5 M NaOH, by gas chromatography according to Visscher and van Gernerden (1991); a Sukelpak S column was used instead of a Porapak column. Before MMPA was determined sulfide was removed by adding 50 µl HCl (1 N) to the sample (1 ml) and gassing with N₂ for 5 min; cells

Abbreviations: DMSP, dimethylsulfoniopropionate; DMS, dimethyl sulfide; MMPA, 3-*S*-methylmercaptopropionate

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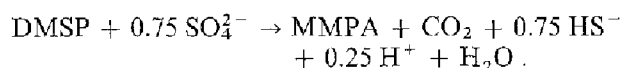
were removed by centrifugation. MMPA was analyzed on a Waters high-performance liquid chromatography system equipped with a 10 cm μ Bondapak C18 column (Waters Associates, Millford, Mass., USA) and a refractometric index detector. The flow rate of the mobile phase (10% v/v methanol in a phosphate buffer [$\text{H}_3\text{PO}_4/\text{K}_2\text{H}_2\text{PO}_4 = 9.8/1.4 \text{ g l}^{-1}$]) was 1.0 ml min^{-1} . Cell carbon was determined according to Heijthuisen and Hansen (1989a).

Chemicals

DMSP was synthesized from acrylate and DMS according to Chambers et al. (1987). MMPA was obtained by alkaline hydrolysis of its methyl ester (Aldrich, Steinheim, Germany).

Results and discussion

During growth strain PM4 formed 1.0 mol of MMPA and 0.58 mol of sulfide per mol DMSP. The identity of the organic compound formed from DMSP was established by cochromatography (HPLC) with authentic MMPA and by proton NMR (data not shown). MMPA was not further degraded to 3-mercaptopropionate. The specific growth rate was 0.021 h^{-1} (t_d 33 h); the growth was only exponential in the early growth phase (24–60 h, if a 1% inoculum was used). The amount of cell carbon produced was 0.24 mol per mol of DMSP, corresponding to a biomass of approx. 5.8 g mol^{-1} on the assumption of a cell carbon content of 50%. The specific growth rate and the amount of cell carbon produced per mol on DMSP are slightly lower than on betaine (0.033 h^{-1} and 0.31 mol/mol of betaine; Heijthuisen and Hansen 1989b). The degradation of DMSP by strain PM4 can be described by the following equation:



At pH 6.9 and initial DMSP concentrations of 10 to 20 mM strain PM4 degraded only 4–5 mM of the substrate. At pH 7.2 strain PM4 could degrade up to 8 mM of DMSP. The incomplete degradation at lower pH is probably due to the inhibitory effect of the hydrogen sulfide in the medium. Brysch et al. (1987) showed that *Desulfobacterium autotrophicum* does not grow at sulfide concentrations of 5 to 10 mM.

Kiene and Taylor (1988a, b) showed that in anoxic marine sediments DMSP is degraded by two different routes. Mercaptopropionate was a detectable intermediate of the demethylation pathway; from the fact that added MMPA was degraded to mercaptopropionate at a rate similar to the demethylation of DMSP to mercaptopropionate it was concluded that MMPA is an intermediate in this pathway. Our pure culture studies with *Desulfobacterium* sp. strain PM4 show that MMPA is indeed a product of DMSP breakdown. This strain is the first example of an anaerobic organism that is able to demethylate DMSP. An analysis of the quantitative role of this type of organisms in DMSP demethylation in marine sediments is subject of further work.

Betaine is metabolized to dimethylglycine by many acetogenic bacteria, such as for example *Eubacterium limosum* (Heijthuisen and Hansen 1990); it was suggested that such bacteria may also be involved in DMSP demethylation (Kiene and Taylor 1988a). Whether marine acetogenic bacteria exist that demethylate DMSP remains to be established.

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